Br. J. Pharmac. (1981), **72**, 13–15

UNEXPECTED ANTAGONISM IN THE RAT VAS DEFERENS BY BENZOMORPHANS WHICH ARE AGONISTS IN OTHER PHARMACOLOGICAL TESTS

MAUREEN G.C. GILLAN, HANS W. KOSTERLITZ & JACQUES MAGNAN

Unit for Research on Addictive Drugs, University of Aberdeen, Marischal College, Aberdeen AB9 1AS

The benzomorphans, ethylketazocine, bremazocine and MR 2034 are pure agonists in the guinea-pig ileum and mouse vas deferens but are competitive antagonists without agonist activity in the rat vas deferens.

Introduction Compared with the mouse vas deferens and the guinea-pig ileum, the rat vas deferens has a different pattern of sensitivity to the inhibitory actions of natural and synthetic opiates and of endogenous opioid peptides and their analogues. Whereas β-endorphin has been found to have similar potencies in all three preparations, methionine-enkephalin, D-Ala^2-D-Leu^5-enkephalin, morphine and etorphine are 2 to 3 orders of magnitude more potent in the guinea-pig ileum and mouse vas deferens than in the rat vas deferens (Lemaire, Magnan & Regoli, 1978; Schulz, Faase, Wüster & Herz, 1979). It is of particular interest that compounds with known partial agonist activity become pure antagonists in the rat vas deferens (Wüster, Schulz & Herz, 1980). We now describe an analysis of benzomorphans which have been classified as pure κ-agonists. In the non-dependent dog, they produce a pattern of effects different from those caused by morphine or other μ-agonists (Martin, Eades, Thompson, Huppler & Gilbert, 1976); in the morphine-dependent dog, one of these compounds, ethylketazocine is a pure strong agonist of the κ-type which neither precipitates nor suppresses morphine abstinence (Gilbert & Martin, 1976). In agreement with these observations, it was found that in the mouse vas deferens and guinea-pig ileum, their agonist action requires more naloxone for their reversal than for that of μ-agonists (Hutchinson, Kosterlitz, Leslie, Waterfield & Terenius, 1975). Their pharmacological pattern is compared with that of μ- and δ-agonists.

Methods Vasa deferentia from hooded rats of the Aberdeen colony (250 to 350 g) were prepared as previously described (Lemaire *et al.*, 1978). The vasa were dissected out, cleaned and mounted in siliconized 3 ml organ baths. The bath fluid was Krebs solution of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.55, KH_2PO_4 1.6, $MgSO_4$ 1.18, $NaHCO_3$ 25, glucose 11, and mepyramine maleate 0.00013; it was bubbled with 95% O_2 and 5% CO_2. After equilibration for 30 min, longitudinal contractions were evoked by field stimulation through Pt-electrodes at the upper and lower ends of the bath. The parameters of stimulation were trains of stimuli consisting of 2 to 3 pulses at intervals of 200 ms and repeated at 0.1 Hz; the pulse duration was 0.5 to 1 ms and the voltage was adjusted to give contractions which were 80 to 90% maximal. The contractions were recorded isometrically, with a resting tension of 1 g.

The potencies of the agonists were obtained from dose-response curves by calculating the concentration of the drug that reduced the height of contractions by 50% (IC_{50}). The affinities of the antagonists were expressed as the equilibrium dissociation constant, K_e (Arunlakshana & Schild, 1959).

The peptides and drugs used were: D-Ala^2-D-Leu^5-enkephalin (Dr S. Wilkinson, Wellcome Research Laboratories), D-Ala^2-MePhE^4-Gly-ol^5-enkephalin (Dr B. A. Morgan, Reckitt & Colman), porcine β-endorphin (Dr R. Guillemin), bremazocine hydrochloride (Dr D. Römer, Sandoz), ethylketazocine (Dr W. Michne, Sterling-Winthrop), MR 2034 as the tartrate ((−)-(1R,5R,9R,2″S)-5,9-dimethyl-2′-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan) and MR 2266 as the free base ((−)-α-5,9-diethyl-2′-hydroxy-2-(3-furylmethyl)-6,7-benzomorphan) (Dr H. Merz, C. H. Boehringer Sohn), naloxone hydrochloride (Endo Laboratories) and normorphine (Wellcome Foundation).

Results The IC_{50} values obtained in the rat vas deferens were as follows: for the μ-agonists, normorphine and D-Ala^2-MePhe^4-Gly-ol^5-enkephalin, 2068 ± 98 nM (n = 21) and 410 ± 25 nM (n = 17), respectively, for the δ-agonist, D-Ala^2-D-Leu^5-enkephalin, 312 ± 13 nM (n = 72) and for porcine β-endorphin, equipotent at μ- and δ-receptors, 23.8 ± 1.9 nM (n = 16). In the vas deferens of the mouse the IC_{50} values were considerably smaller than in the rat; they were for normorphine, D-Ala^2-MePhe^4-Gly-ol^5-enkephalin and D-Ala^2-D-Leu^5-enkephalin 400 ± 31 nM

0007-1188/81/010013-03 $01.00

Table 1 Equilibrium dissociation constants in the rat vas deferens

Compounds	Equilibrium dissociation constants (K_e, nM) Normorphine	D-Ala²-MePhe⁴-Gly-ol⁵-enkephalin	D-Ala²-D-Leu⁵-enkephalin	Porcine β-endorphin
Agonists in G.p.i. and M.v.d.				
Ethylketazocine	59 ± 6.6	82 ± 4.4	206 ± 29	332 ± 18
MR 2034	18.6 ± 2.1	18.2 ± 1.0	301 ± 13	67
Bremazocine	1.8 ± 0.5	2.7 ± 0.1	8.2 ± 0.7	5.3 ± 1.2
Antagonists in G.p.i. and M.v.d.				
Naloxone	6.3 ± 0.4	7.5 ± 0.8	33.7 ± 1.3	14.7 ± 1.2
MR 2266	7.6 ± 0.4	8.8 ± 0.8	37.2 ± 7.7	14.6 ± 2.4

The values are the means ± s.e.mean of 3–4 experiments, except for MR 2034 against β-endorphin. The data were obtained with the (−)-isomers; the (+)-isomers of ethylketazocine, MR 2034 and MR 2266 were inactive. The slopes of the regression line of log (DR − 1) on log concentration of antagonist varied between 0.8 and 1.1. G.p.i. = Guinea-pig ileum; M.v.d. = mouse vas deferens

(n = 32), 43.0 ± 7.9 nM (n = 3) and 0.54 ± 0.09 nM (n = 15), respectively.

The benzomorphans, ethylketazocine, MR 2034 and bremazocine, which are pure agonists in the mouse vas deferens and in the guinea-pig ileum, were found to have no agonist activity in the rat vas deferens but to be competitive antagonists of the agonists tested. They were more potent against the μ-agonists normorphine and D-Ala²-MePhe⁴-Gly-ol⁵-enkephalin than against the δ-agonist D-Ala²-D-Leu⁵-enkephalin, an activity pattern similar to that of naloxone or MR 2266 which are antagonists also in the guinea-pig ileum and mouse vas deferens (Table 1). In the mouse vas deferens, naloxone had a K_e value of 1.64 ± 0.23 nM (n = 3) against D-Ala²-MePhe⁴-Gly-ol⁵-enkephalin; K_e values against normorphine of 1.8 nM and against D-Ala²-D-Leu⁵-enkephalin of 32 nM have been reported (Kosterlitz & Paterson, 1980).

Discussion On the evidence available at present there is no unequivocal explanation for the finding that three benzomorphans which have potent agonist but no antagonist activity in the guinea-pig ileum and mouse vas deferens (Hutchinson *et al.*, 1975), are in the rat vas deferens antagonists of varying potency without any agonist activity. It may be speculated that the receptor sites with which the benzomorphans interact are similar in the vasa deferentia of the two species but that in the rat the benzomorphans cannot produce the conformational changes required for agonist action. There is so far no direct information as to which subtype of opiate receptor the benzomorphans bind in the rat vas deferens. In homogenates of guinea-pig brain, tritiated κ-agonists bind saturably and specifically to the κ-binding site from which ligands selective for μ- and δ-binding sites displace them only in high concentrations. On the other hand, κ-agonists readily displace μ-agonists from the μ-binding site (Kosterlitz & Paterson, 1980). Since, in the rat vas deferens, the benzomorphans antagonize the selective μ-agonists more readily than the selective δ-agonist or the μ plus δ-agonist β-endorphin, a working hypothesis is proposed that the antagonist action of the three benzomorphans is due to interaction at the μ-site. Binding assays on the rat vas deferens will have to decide whether this concept is correct and, in particular, whether there are typical κ-binding sites in this tissue.

Supported by grants from the Medical Research Council and the U.S. National Institute on Drug Abuse. J.M. has a fellowship from the Canadian Medical Research Council. Acknowledgement is made of generous gifts of the compounds mentioned in Methods.

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

GILBERT, P.E. & MARTIN, W.R. (1976). The effects of morphine- and nalorphine-like drugs in the nondependent, morphine-dependent and cyclazocine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.*, **198**, 66–82.

HUTCHINSON, M., KOSTERLITZ, H.W., LESLIE, F.M., WATERFIELD, A.A. & TERENIUS, L. (1975). Assessment in the guinea-pig ileum and mouse vas deferens of benzomor-

phans which have strong antinociceptive activity but do not substitute for morphine in the dependent monkey. *Br. J. Pharmac.*, **55**, 541–546.

KOSTERLITZ, H.W. & PATERSON, S.J. (1980). Characterization of opioid receptors in nervous tissue. *Proc. R. Soc. B*, **210**, 113–122.

LEMAIRE, S., MAGNAN, J. & REGOLI, D. (1978). Rat vas deferens: a specific bioassay for endogenous opioid peptides. *Br. J. Pharmac.*, **64**, 327–329.

MARTIN, W.R., EADES, C.G., THOMPSON, J.A., HUPPLER, R.E. & GILBERT, P.E. (1976). The effects of morphine- and nalorphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.*, **197**, 517–532.

SCHULZ, R., FAASE, E., WÜSTER, M. & HERZ, A. (1979). Selective receptors for β-endorphin on the rat vas deferens. *Life Sci.*, **24**, 843–850.

WÜSTER, M., SCHULZ, R. & HERZ, A. (1980). Opioid agonists and antagonists: Action on multiple opiate receptors. In *Endogenous and Exogenous Opiate Agonists and Antagonists*. ed. Way, E.L. pp. 75–78. New York: Pergamon Press.

(*Received October 27, 1980.*)